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Phylogeny and historical biogeography of *Isodon* (Lamiaceae): Rapid radiation in south-west China and Miocene overland dispersal into Africa

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ABSTRACT

Rapid organismal radiations occurring on the Qinghai–Tibetan Plateau (QTP) and the mechanisms underlying Asia–Africa intercontinental disjunctions have both attracted much attention from evolutionary biologists. Here we use the genus *Isodon* (Lamiaceae), a primarily East Asian lineage with disjunct species in central and southern Africa, as a case study to shed light upon these processes. The molecular phylogeny and biogeographic history of *Isodon* were reconstructed using sequences of three plastid markers, the nuclear ribosomal internal transcribed spacer (nrITS), and a low-copy nuclear gene (*LEAFY* intron II). The evolution of chromosome numbers in this genus was also investigated using probabilistic models. Our results support a monophyletic *Isodon* that includes the two disjunct African species, both of which likely formed through allopolyploidy. An overland migration from Asia to Africa through Arabia during the early Miocene is proposed as the most likely explanation for the present disjunct distribution of *Isodon*. The opening of the Red Sea in the middle Miocene may appear to have had a major role in disrupting floristic exchange between Asia and Africa. In addition, a rapid radiation of *Isodon* was suggested to occur in the late Miocene. It corresponds with one of the major uplifts of the QTP and subsequent aridification events. Our results support the hypothesis that geological and climatic events play important roles in driving biological diversification of organisms distributed in the QTP area.

1. Introduction

Orogenic and environmental processes play a major role in organismal evolution (Richardson et al., 2001; Mao et al., 2012). The geological configuration of the Asian plate was suggested to be greatly shaped by the India–Eurasia collision and the Arabia–Eurasia collision (Yin, 2010). The former triggered the formation of the Qinghai–Tibetan Plateau (QTP), uplift of which were reported to promote the rapid radiation of numerous organisms (Liu et al., 2006; Sun et al., 2012), while the latter also served as a bridge for floral and faunal exchange between Africa and Asia (Stewart and Disotell, 1998; Zhou et al., 2011). Although both tectonic events probably altered species distribution patterns dramatically, few studies have addressed the questions directly, or tested hypotheses involving the two processes using appropriate taxa.

The Qinghai–Tibetan Plateau (QTP) complex, sometimes called the 'roof of the world', harbors extremely rich species diversity and endemism (Wu, 1988). Elucidating the mechanisms driving plant speciation in this region is of great interest. Organismal diversification in this region has possibly been elevated, at least in part, by the severe alteration of topography and accompanying climate change that ensued since the uplift of the QTP during the Miocene and Quaternary (Liu et al., 2006; Sun et al., 2012). Rapid

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radiation, as inferred by short internal branch lengths (Richardson et al., 2001) and large polytomies (low DNA sequence divergence and poor resolution), has been reported for many plant groups such as Saxifragales (Jian et al., 2008) and Malpighiales (Xi et al., 2012). Research investigating rapid radiations of species-rich plant groups in the QTP area may thus enrich our understanding of the evolutionary origins of biodiversity (Erkens et al., 2012).

Intercontinental disjunct distributions between closely related species are a remarkable feature of angiosperm biogeography (Raven and Axelrod, 1974). Disjunct distribution patterns from the Northern Hemisphere, especially the Eastern Asia–Eastern North America disjunctions, have been investigated extensively (Wen, 1999; Nie et al., 2006). In contrast, mechanisms responsible for Southern Hemisphere disjunctions (e.g. Asia–Africa disjunction) have received far less attention (e.g. Yuan et al., 2005; Zhou et al., 2011). The (Africa–) Arabia–Eurasia collision was considered equally important to the India–Eurasia collision (Yin, 2010) and might have played a significant role in floral and faunal exchange between Africa and Asia.

Overland migration between Africa and Eurasia across the Arabian Peninsula has been proposed for *Hoplobatrachus* Peters, 1863 (Amphibia: Ranidae) through a connection between the Arabian Peninsula and Asia in the Miocene (Kosuch et al., 2001); *Lychnis* L. (Caryophyllaceae) from Eurasia to the Ethiopian highlands via the Arabian Peninsula (Popp et al., 2008); and *Uvaria* L. (Annonaceae), which has been associated with the 'out-of-Africa' dispersal and radiation of primates during the Miocene (Zhou et al., 2011). Other studies (e.g. Kulju et al., 2007) have also discussed the possibility of overland dispersal between Africa and Asia. However, competing hypotheses also exist: the 'Indian rafting' hypothesis (during the Cretaceous) (Conti et al., 2002); dispersal of high-latitude boreotropical floras (from the late Paleocene to middle Eocene) (Davis et al., 2002); and trans-oceanic long-distance dispersal (Yuan et al., 2005) are three other mechanisms commonly invoked to explain Asia–Africa disjunctions.

Isodon (Schrad. ex Benth.) Spach (tribe Ocimeae Dumort.; subfamily Nepetoideae) is a genus of ca. 100 species in Lamiaceae (Harley et al., 2004). The genus is distributed primarily in tropical and subtropical Asia, with a center of species diversity (ca. 70%) in the Hengduan Mountains region (HMR; southeastern corner of the QTP) of south-west China. The genus also ranges west to Afghanistan and Pakistan, with a further two disjunct species *I. ramosissimus* (Wall. ex Benth.) Murata and *I. schimperi* (Vatke) J.K. Morton in central and southern Africa. *Isodon* includes shrubs, subshrubs, and perennial herbs with paniculate inflorescences composed of many-flowered cymes. The corollas of *Isodon* species can differ in size, tube length, color, and the color of spots on the lips, possibly as an adaptive response to pollinators. The majority of *Isodon* species occurring in the HMR region occupy cold and arid highland habitats, but a few species extend to humid and warm tropical regions. In contrast, the two African *Isodon* species grow in more humid habitats, such as the margins of evergreen forests or in riparian zones (pers. obs; Li, 1988).

Li (1988) monographed the genus in China, dividing it into four sections based on morphological characters such as inflorescence density and the shape of the fruiting calyx. Later, the two African species were added (Ryding, 1993; Morton, 1998), but their molecular phylogenetic position within *Isodon* has not been tested. In addition, there was apparently at least one chromosome number shift in *Isodon*: the African species *I. ramosissimus* has a chromosome number of $2n = 42$ and a base chromosome number proposed to be $x = 7$ (Morton, 1962, 1993), or $x = 14$ (Yamashiro et al., 2005). In contrast, the Asian species have numbers of $2n = 24$ or $2n = 36$, with a suggested base chromosome number of $x = 12$ (Jin and Sha, 2004; Yamashiro et al., 2005; Huang, 2011).

Our previous research focused mainly on the relationship between *Isodon* and related genera and the two African species were not involved in (Zhong et al., 2010). Accordingly, in this study, we aim to obtain a more comprehensive phylogenetic reconstruction of *Isodon* with the two African species included to illuminate the evolution of this species-rich genus. We are also using *Isodon* as a case study to understand better apparently rapid species radiations in the Hengduan Mountains region of south-west China and the possible mechanisms triggering Asia–Africa disjunctions. Molecular phylogenetic approaches are used to reconstruct the possible historical biogeography of *Isodon* and investigate the broad topics mentioned above, as well as to answer the following specific questions:

1. Does the radiation of *Isodon* correlate with the suggested geological hypotheses concerning the QTP region and associated climate change?
2. What is the phylogenetic position of the two endemic African species with ostensibly aberrant chromosome number?
3. If the two endemic African species are confirmed to be members of *Isodon*, what mechanisms might be responsible for this Asia–Africa disjunction?

2. Materials and methods

2.1. Taxon sampling

The classification of *Isodon* largely follows Li (1988), but eight species [*Isodon anisochilus* C.Y. Wu, *I. brachythyrsus* C.Y. Wu et H.W. Li, *I. forrestii* var. *intermedius* C.Y. Wu et H.W. Li, *I. kunmingensis* C.Y. Wu et H.W. Li, *I. pluriflorus* C.Y. Wu et H.W. Li, *I. polystachys* (Sun ex C. H. Hu) C.Y. Wu et H.W. Li, *I. setschwanensis* var. *yungshengensis* C.Y. Wu et H.W. Li and *I. taliensis* (C.Y.Wu) Hara] were treated as distinct taxa following Wu and Li (1977) in order to represent the species diversity of this genus better. *Isodon umbrosus* (Maxim.) H. Hara and its varieties, which were not included in Li (1988), were added following Flora of Japan (Murata and Yamazaki, 1993) and the inclusion of *I. oreophilus* (Diels) A.J. Paton et Ryding follows Zhong et al. (2010). *Isodon* sp. aff. *flavidus* (Hand. -Mazz.) H. Hara was treated as a separate taxonomic entity due to its allopatric distribution with respect to *I. flavidus* (Hand. -Mazz.) H. Hara and the samples labelled *I. eriocalyx* (Dunn) Kudô 214 and *I. henryi* (Hemsl.) Kudô 232 were included as putative hybrids. A further six specimens that could not be identified accurately to known taxa were also treated as separate *Isodon* OTUs (operational taxonomic units) in the analyses.

Samples of DNA for the two African species were obtained from the DNA Bank of the Royal Botanic Gardens, Kew. In total, 73 accessions representing 71 species of *Isodon* were included in this study. The complete list of voucher specimen numbers, collection localities, distribution codes, available chromosome numbers, and GenBank accession numbers of samples used are given in Supplementary Table S1. nrITS sequences (Supplementary material, Table S2) for 26 *Isodon* species (based on different vouchers) sequenced in our previous study were also added to the present data set. Voucher specimens of samples from Africa and Japan are deposited at the herbarium of the Royal Botanic Gardens, Kew (K) and the herbarium of Botanical Gardens in Tohoku University of Japan (TUS), respectively. Others are deposited at Laboratory of Plant Phylogenetics and Conservation, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences (XTBG). To augment our sampling, we also downloaded 21 nrITS sequences from other Ocimeae genera from GenBank (Supplementary material, Table S2). Similarly, we also downloaded 39 *rpl*32–*trn*L sequences from various genera in Lamiaceae (Supplementary material, Table S3).

Based on the work of Zhong et al. (2010), our outgroup taxa included seven species representing six genera in Ocimeae for the cpDNA data set and 22 species representing ten genera in Ocimeae for the nrITS data set. Since the Yule model used in our molecular dating analysis assumes more or less even sampling throughout the tree (Velasco, 2008), we sequenced *rpl*32–*trn*L from three additional species of *Elsholtzia* Willd. for this study to make the Elsholtzieae (with only two published accessions available) better represented.

2.2. DNA extraction, PCR and DNA sequencing

Total genomic DNA was extracted from leaf tissue using a modified cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1987). We sequenced three chloroplast (cpDNA) intergenic spacer regions (*trn*D–*trn*T, *psb*A–*trn*H, and *rpl*32–*trn*L). Two nuclear DNA fragments: ribosomal internal transcribed spacers (nrITS) and the second intron of the *LEAFY* gene (part sequences from the second and third exons) were also used. The primers and corresponding annealing temperatures used in this study are listed in Supplementary Table S4. To amplify and sequence the second intron of *LEAFY*, *Isodon*-specific primers were designed using the degenerate primers LFsxl-2 and LFtxr (Frohlich and Meyerowitz, 1997) as a starting point.

Polymerase chain reaction conditions, product purification, cloning and cycle sequencing followed protocols described elsewhere (Li et al., 2011). Multiple bands obtained from *LEAFY* amplification were separated by excision and elution from the gels using an OMEGA quick Gel Extraction Kit (OMEGA) and then used for cloning. Direct sequencing of nrITS PCR products from universal primers produced "clean" sequences, except for *Isodon eriocalyx* 214, *I. medilungensis* (C.Y. Wu et H.W. Li) H. Hara, *I.* sp. 2 and *I. dawoensis* (Hand. -Mazz.) H. Hara. Those sequences were obtained through cloning, with at least five positive clones sequenced for each individual. We could not obtain the nrITS sequence data from *I. henryi* 232.

For the *LEAFY* intron II fragment, two distinct copies with different sizes (A and B) were found in four species of *Isodon*, consistent with the findings of Aagaard et al. (2005). There might be selective amplification of the two copies, as copy A was obtained in all of the ingroup, while copy B was only obtained from four species of *Isodon* and with an almost identical sequence. It is possible that copy A was easier to be detected by our specific primers. Copy B from the four species of *Isodon* was used as an outgroup because of the great difficulties in alignment between ingroup and outgroup taxa.

2.3. Sequence alignment and phylogenetic analyses

DNA sequences were aligned with the MAFFT algorithm (Katoh et al., 2005) in SATé v2.25 (Liu et al., 2012) using default settings and edited manually in MEGA v5.0 (Tamura et al., 2011). Alignment gaps were treated as missing data. For multiple clones from each individual, a single representative sequence was chosen randomly for phylogenetic analysis if all the clones formed a well-supported clade in the preliminary analysis. But multiple sequences were retained if they grouped with different accessions. Maximum parsimony (MP) analyses were conducted using the heuristic search option in PAUP* v4.0b10 (Swofford, 2003). The parameters are 1000 random addition sequence replicates, tree-bisection-reconnection (TBR) swapping, MulTrees on, with 1000 trees saved from each random addition sequence replicate. Bootstrap support values (BS) for the internal nodes were obtained with 100 bootstrap replicates using the same settings presented above. We used jModelTest v0.11 (Posada, 2008) to select the best-fitting nucleotide substitution models for analyses of Bayesian inference (BI) and BEAST according to the Akaike information criterion (AIC; Akaike, 1974). Nucleotide models were as follows: cpDNA, GTR+I; nrITS, GTR+G; *LEAFY* exons: K80+I; *LEAFY* intron: GTR+G. BI analyses were performed using the program MrBayes v3.12 (Ronquist and Huelsenbeck, 2003). Two runs were conducted in parallel with four Markov chains, with each running for 2,000,000 generations and sampled every 100th generation. Examination of the log-likelihood values suggested that stationarity was reached in about 50,000 generations. Thus, the first 200,000 generations (10%) were discarded to make sure the burn-in period was sufficiently long. For purposes of the results and discussion in this paper, clades with posterior probability values of 0.50–0.79 were considered weakly supported, 0.80–0.94 moderately supported, and 0.95–1.00 strongly (well-) supported.

In this study, incongruence between different gene trees was identified in two ways: (1) we compared individual gene topologies and considered topologies incongruent when conflicting nodes were supported by at least 70% bootstrap support (BS) and 0.9 Bayesian posterior probability in one of the two gene trees; or (2) we conducted the incongruence length difference (ILD) test (Farris et al., 1995). When incongruence is observed between different gene trees, the trees can then be used to calculate a network which represents potential hybridization events (Russell et al., 2010). For the incongruent position of the African lineage found between the cpDNA and nrITS trees (see results), the phylogenetic network method was employed using both the 70% and 90% (for comparison) Bayesian consensus trees in Dendroscope v3.0 (Huson and Scornavacca, 2012).

2.4. Molecular dating of Isodon

As there are no known fossils for *Isodon*, molecular dating in this study relies on fossils from related clades in Lamiaceae, such as the early Eocene fossil hexacolpate putative pollen of *Ocimum* L. identified by Kar (1996) and the early–middle Oligocene fruit fossil of *Melissa* L. (Reid and Chandler, 1926; Martínez-Millán, 2010). These two fossils have also been employed in recent phylogenetic studies of Lamiaceae (Drew and Sytsma, 2012, 2013). Those authors placed the hexacolpate pollen described by Kar (1996) as a putative *Ocimum* at the crown of Nepetoideae (as opposed to the crown of Ociminae) and the *Melissa* fossil at the stem of this genus. We agree with this conservative placement.

Divergence times were estimated using a Bayesian method implemented in the program BEAST v1.72 (Drummond et al., 2006; Drummond and Rambaut, 2007). For all BEAST analyses, we implemented a Yule process speciation prior and both the uncorrelated exponential (UCED) and lognormal (UCLN) relaxed clock model of rate change were conducted. Bayes factors calculated with Tracer v1.5 (Rambaut and Drummond, 2007) were used to compare the results of UCED and UCLN. Two separate runs were conducted, each with 20,000,000 generations sampled every 1000 generations and the resulting log files and trees from each run were combined with the program Log Combiner v1.72. Tree Annotator v1.72 was used to summarize the set of post burn-in (10%) trees and their parameters. The combined log file was checked using Tracer v1.5 to ensure the effective sample sizes (ESS) were all above 200. Finally, the maximum clade credibility (MCC) chronogram was visualized using the program FigTree v1.3.1 (Rambaut and Drummond, 2010).

Estimation of *Isodon* divergence times was done in two steps. Firstly, stem and crown ages of *Isodon* was estimated using the *rpl*32–*trn*L data set which included 61 species representing all three tribes (Elsholtzieae, Mentheae and Ocimeae) in Nepetoideae (Harley et al., 2004) and four outgroups (Supplementary Table S3). In this initial analysis, only 12 species representing the major clades of *Isodon* were included, as the Yule process assumes that

the sampling was even throughout the tree (Velasco, 2008). The GTR+G model of substitution was suggested for this data set. The lognormal prior distribution, was used for the fossil calibrations, as it is perhaps the most appropriate distribution for summarizing paleontological information (Ho and Phillips, 2009). Prior parameters for the two fossil calibrations in this study followed Drew and Sytsma (2012), but we did not employ temporal constraints on the root of the tree.

Secondly, we conducted three detailed analyses within *Isodon* using a combined cpDNA, nrITS, and *LEAFY* intron II data set, respectively. It has been suggested that relaxed clock dating methods are unable to reconstruct the pattern of molecular rate change accurately with only one internal calibration (Sauquet et al., 2012). Three crown ages (see Section 3) within *Isodon* obtained from our first analysis were used as 'secondary calibration' points, using a lognormal prior distribution. Through adjusting the value of the mean and standard deviation, the 95% highest posterior density (HPD) of main nodes yielded from the detailed analyses was made to be consistent with that obtained from our first analysis.

2.5. Diversification rates of Isodon

The diversification rate change over time within *Isodon* was explored using several programs based on R applications. All of our three data sets (cpDNA, nrITS and *LEAFY* intron II) were used for analyses. Firstly, we used a maximum likelihood method as implemented in LASER v 2.4-1 (Rabosky, 2006) to determine whether diversification rates of *Isodon* have changed over time. The test statistic for diversification rate-constancy was calculated as: $\Delta AIC_{RC} = AIC_{RC} - AIC_{RV}$, where AIC_{RC} was the AIC score for the best fitting rate-constant diversification model, and AIC_{RV} was the AIC for the best fitting rate-variable diversification model. A positive value for ΔAIC_{RC} indicates that the data are best explained by a rate-variable model of diversification. Two rate-constant (pure birth and birth–death model) and three rate-flexible diversification models (a logistic density-dependent speciation rate, an exponential density-dependent and a yule2rate model) were evaluated in our study. Secondly, the Relative Cladogenesis (RC) test was performed in GEIGER v 2.01 (Harmon et al., 2008) to detect shifts of diversification rates within *Isodon*, using the MCC chronogram generated from BEAST analyses. Bonferroni correction was used to adjust the P-values. Thirdly, we calculated the semi-logarithmic lineage through time (LTT) plots by APE v 3.1-1 (Paradis et al., 2004). We used 3000 trees chosen randomly from the BEAST tree-chains to generate the confidence intervals.

2.6. Ancestral area reconstructions

Five biogeographic regions were delimited, based on species distributions (Morton, 1962; Wu and Li, 1977; Li, 1988; Friis and Vollesen, 2005): (A) QTP and adjacent regions, including the QTP, northeastern India, Nepal, Bhutan, Afghanistan, Pakistan, and Yunnan Plateau; (B) tropical Asia, including southern India, Sri Lanka, Bangladesh and other tropical areas in China and southeastern Asia; (C) eastern Asia, including areas of south, central, north, east and northeast China, Korea and parts of Russia; (D) Japan; (E) tropical Africa, including Equatorial Guinea, Cameroon, Burundi, Tanzania, Sierra Leone, southern Zimbabwe, southern Sudan, Uganda, and Ethiopia.

Ancestral area reconstructions (AAR) were conducted using both the Statistical Dispersal–Vicariance (S-DIVA) approach (Yu et al., 2010) and likelihood analysis under the dispersal–extinction–cladogenesis (DEC) model (Ree et al., 2005; Ree and Smith, 2008). Analyses were implemented in RASP v2.1 (Yu et al., 2011) and Lagrange, respectively. For the latter analysis, we considered the connection between Asia and Africa to have been present from ca. 20 Ma based on previous studies (Rögl, 1998, 1999) and migration between eastern Asia and Japan was allowed since ca. 5 Ma (Kizaki and Oshiro, 1977). For both S-DIVA and Lagrange approach, we conducted two analyses with the maximum area number at each node set as 2 and 3, respectively, since no species included in our study occurred in more than three of our defined biogeographic regions.

AAR analyses were conducted using both cpDNA and nrITS data set. However, the analysis using the *LEAFY* intron II data set was not attempted since two homoeologous copies were obtained from some putative hybrids, including the two African species, but multiple copies were not present in all species. *Hanceola sinensis* (Hemsl.) Kudô and *Orthosiphon wulfenioides* (Diels) Hand.-Mazz. were chosen as outgroups, based on the results from our molecular phylogenetic reconstruction. Since relationships among species of *Isodon* were not fully resolved in this study, AARs could not be estimated unambiguously within all the major clades recovered. Thus, only biogeographic events of nodes or branches with strongly-supported posterior probability (above 0.95) were inferred.

2.7. Evolution of chromosome numbers

We used the program chromEvol v1.3 (Mayrose et al., 2010) to infer ancestral chromosome numbers in *Isodon*. It estimates changes in chromosome number along a phylogenetic tree by implementing eight likelihood models. Besides gain, loss, and polyploidy, "demi-polyploidization", which represents an increase in chromosome number by the union of a reduced and an unreduced gamete (Mayrose et al., 2010), was also taken into account. A Bayesian consensus tree (constructed from the combined cpDNA matrix), pruned to include only the species with reported chromosome numbers, was used as an input tree. Species with chromosome numbers and the corresponding references are shown in Supplementary Table S1. All clades except the monospecific clade II had species represented for this analysis (see Section 3). Because different chromosome numbers have been reported in *Ocimum basilicum* L. (Paton and Putievsky, 1996; Mukherjee et al., 2005), we only used the most common count for analysis. The program was run under the default parameters using the best-fitting model selected according to the likelihood ratio tests using the Akaike information criterion (AIC).

3. Results

3.1. Phylogenetic analysis

The three cpDNA gene regions were combined since no conflicts were observed among partitions. However, we did not combine the two nuclear data sets as the ILD test suggested significant incongruence between the nrITS and *LEAFY* intron II data sets ($p < 0.01$). Moreover, the cpDNA data set was not combined with either the nrITS or *LEAFY* intron II data set, based on both the results from the ILD test and the incongruent phylogenetic position of the two African species. The aligned lengths of the *psb*A–*trn*H, *trn*D–*trn*T, *rpl*32–*trn*L, nrITS, and *LEAFY* intron II data sets were 465, 1019, 1051, 650 and 2364 bp, respectively. The combined plastid data set was 2535 bp in length.

The monophyly of *Isodon*, including the two African species, was supported strongly by the cpDNA (BS = 98; PP = 1.0; Fig. 1A), nrITS (BS = 77; PP = 1.0; Fig. 1B), and *LEAFY* intron II (BS = 100; PP = 1.0; Fig. 2) trees. *Isodon* was found to contain four main clades. Three of these clades were well-supported Asian lineages, consistent with previous findings (Zhong et al., 2010): an early-branching clade I; a monospecific clade II; and clade III (two well-supported

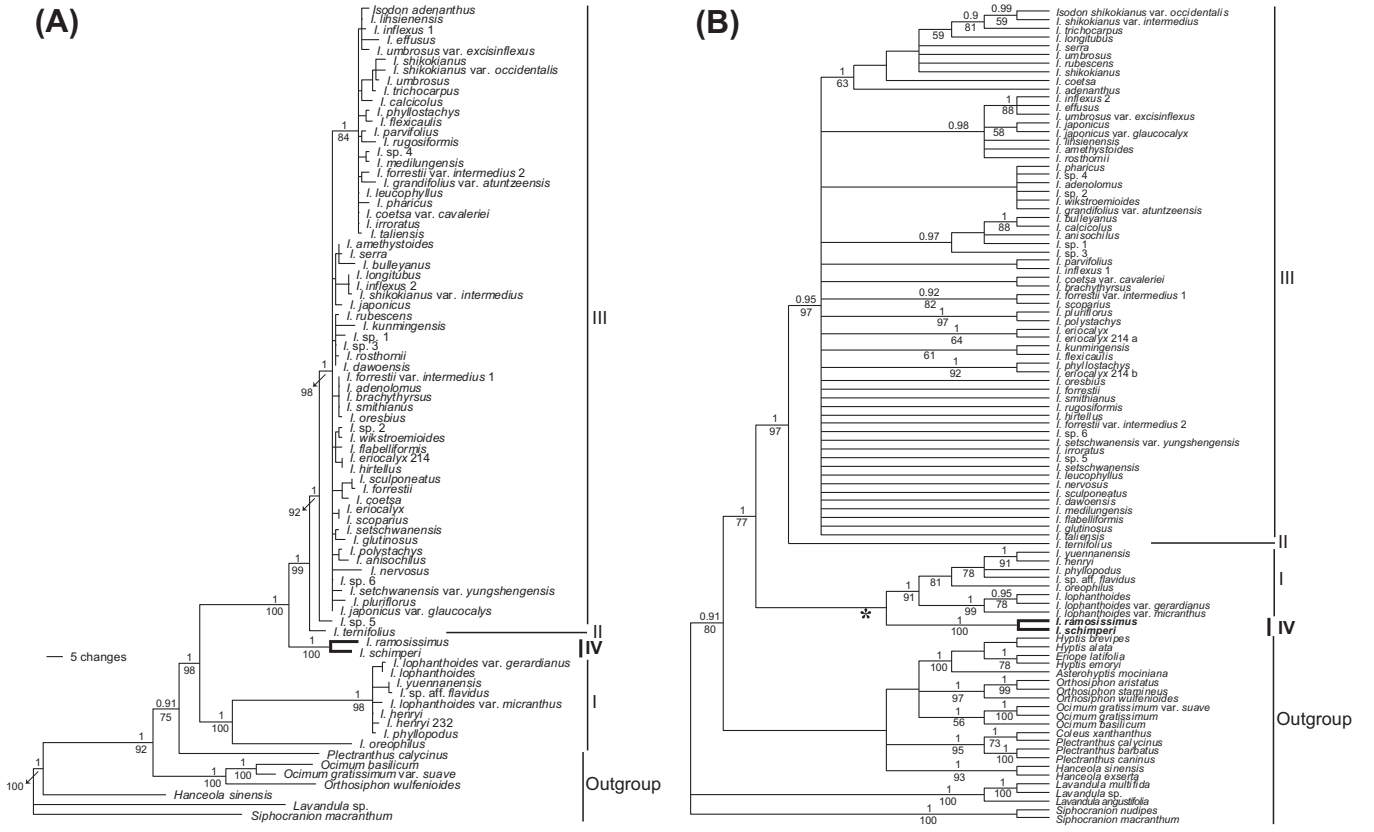

Fig. 1. Fifty percent majority rule consensus trees from Bayesian analysis of combined cpDNA sequence data set (with branch lengths) (A) and nrITS sequence data set (B). Support values of nodes ⩾ 0.9 PP (posterior probability) and 50% BS (bootstrap support) are shown above and below the branches respectively and only those for major nodes are shown in the cpDNA tree. Scale bars indicate expected substitutions. "a" and "b" in the nrITS tree indicate distinct types of sequences isolated from a single individual; branches in bold represent the African clade. * indicates conflict between the parsimony and Bayesian analyses.

subclade III-1 and III-2 in *LEAFY* intron II tree) that includes the remaining Asian species (Figs. 1 and 2). The two species endemic to Africa formed a strongly supported clade (clade IV) in both the cpDNA (BS = 98; PP = 1.0) and nrITS (BS = 100; PP = 1.0) trees. However, the position of the African lineage differed between the analyses using the above two data sets.

The African clade was embedded in Asian clades and shared a common ancestor with clades II and III in the cpDNA tree (BS = 100; PP = 1.0) (Fig. 1A). In the nrITS tree, the African clade was grouped with clade I with weak Bayesian support (PP = 0.71) (Fig. 1B), but was placed as the sister to all other ingroup taxa in the MP analysis (BS < 50%). Nevertheless, it was distant from the clade II–III, a different pattern from the cpDNA tree. The topologies recovered from the Parsimony and Bayesian analyses of the three DNA data sets were generally identical, except for the African clade. Thus, for each data set, only the Bayesian consensus tree was shown, but with both the parsimony bootstrap support and Bayesian posterior probability values indicated.

For the *LEAFY* intron II data set, all the sequences of copy A formed a well-supported clade (BS = 100; PP = 1.0). It provided evidence that these sequences are orthologues of *LEAFY* intron II. Notably, two distinct types of sequences of *LEAFY* intron II (IV-a: BS = 100; PP = 1.0; IV-b: BS = 100; PP = 1.0) were obtained for the two African species. Clade IV-a grouped with clade I, and IV-b appeared as sister to the clade II–III (Fig. 2). However, clade IV-b was placed as the sister to all other ingroup in the MP analysis, which was also found in the analysis of nrITS data set. Within clade III, polytomies were found in all three phylogenetic trees and only the cpDNA tree with branch lengths from the BI analysis was shown (Fig. 1A). The extremely short internal branch lengths were typical of the whole genus. For the two putative hybrids, two distinct types of sequences were obtained from both nrITS and *LEAFY* intron II in *I. eriocalyx* 214. One grouped with *I. eriocalyx* (nrITS: BS = 64; PP = 1.0; *LEAFY* intron II: BS = 54; PP = 0.94) and the other grouped with *I. phyllostachys* (nrITS: BS = 92; PP = 1.0; *LEAFY* intron II: BS = 82; PP = 1.0). However, *I. eriocalyx* 214 appeared as sister to *I. hirtellus* with strong support (BS = 78; PP = 1.0) in the cpDNA tree. Although we could not obtain the nrITS sequence for *I. henryi* 232, two distinct sequences obtained from *LEAFY* intron II for this sample grouped with *I.* sp. aff. *flavidus* and *I. henryi* with strong (BS = 84; PP = 1.0) and moderate (BS < 50%; PP = 0.82) support, respectively.

Results of the phylogenetic network analysis using both 70% and 90% Bayesian consensus trees between cpDNA and nrITS consistently showed reticulation in clade III (Supplementary Fig. S1). Although the analysis using the 70% Bayesian consensus trees might be more likely to suffer from stochastic error, it suggested that the African clade was of hybrid origin between members of clade I and clade II–III. This result was further supported by the gene tree generated from *LEAFY* intron II data set (see above).

3.2. Divergence time estimates

The comparison of Bayes factors that were calculated from UCED and UCLN relaxed clock models indicated that UCED was better than UCLN in all of our BEAST analyses. Thus, we only provided the results under the UCED relaxed clock model. Although the root of the tree was not constrained, divergence times of major nodes of Nepetoideae inferred from the BEAST analysis of the

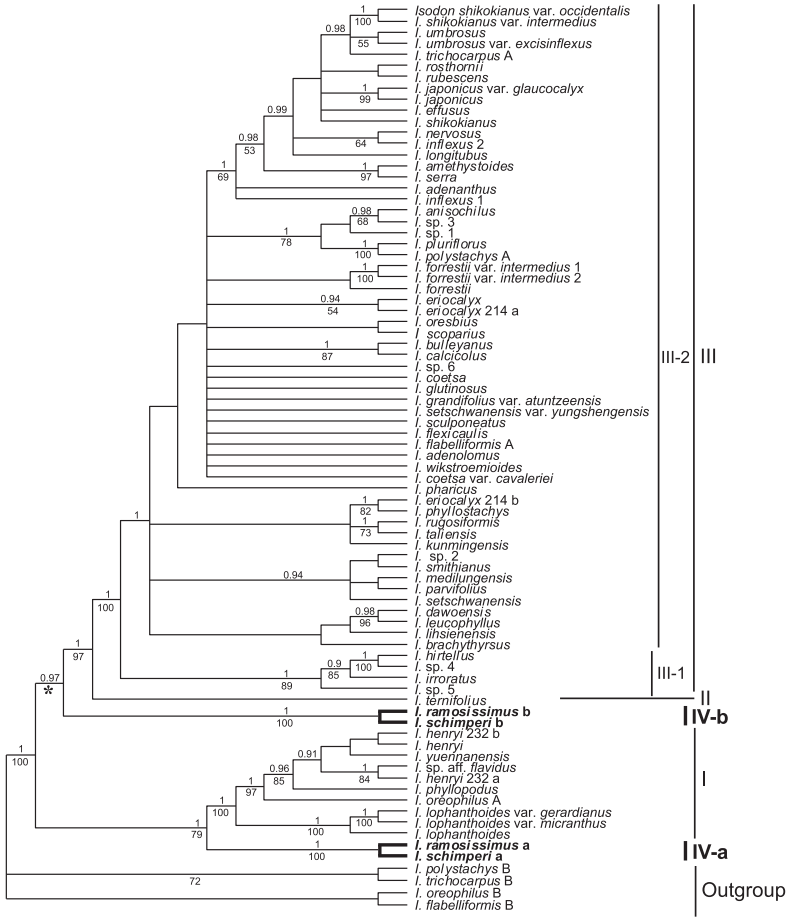

Fig. 2. Fifty percent majority rule consensus tree from Bayesian analysis of *LEAFY* intron II sequence data set. Support values of nodes ⩾ 0.9 PP (posterior probability) and 50% BS (bootstrap support) are shown above and below the branches respectively. "A" and "B" indicate different copies of *LEAFY*, "a" and "b" indicate distinct types of sequences isolated from a single individual; branches in bold represent the African clade.* indicates conflict between the parsimony and Bayesian analyses.

Table 1
Divergence time estimates of BEAST analyses for major nodes of Nepetoideae and *Isodon*.

Node	Age estimated in this study						Age estimated in Drew and Sytsma (2012)	
	cpDNA		ITS		*LEAFY* intron II		cpDNA	
	Mean	95% HPD	Mean	95% HPD	Mean	95% HPD	Mean	95% HPD
C1: Nepetoideae crown	61.36	52.64–74.78	–	–	–	–	57.29	52.4–63.7
C2: *Melissa* stem	32.48	29.56–37.16	–	–	–	–	32.01	29.68–35.63
a: Mentheae crown	45.53	34.9–58.6	–	–	–	–	45.72	40.01–52.08
b: Ocimeae crown	43.04	28.55–57.89	–	–	–	–	43.7	34.32–52.84
c: *Isodon* stem	27.32	17.03–39.84	–	–	–	–	24.90	17.19–32.35
d: *Isodon* crown	19.61	14.66–26.44	19.78	14.15–27.57	21.42	15.10–30.81	–	–
e: Split between II–III and IV	13.61	9.81–18.53	–	–	–	–	–	–
f: Split between II and III	10.76	7.50–14.93	13.20	8.31–19.55	10.26	6.43–15.04	–	–
g: I lineage crown	8.12	3.89–13.58	8.14	3.73–13.77	5.75	2.33–10.43	–	–
h: III lineage crown	9.28	6.26–13.09	9.87	5.91–14.91	7.62	4.70–11.63	–	–
i: Crown of III lineage exclude *I.* sp. 5	7.84	5.14–11.17	–	–	–	–	–	–
j: IV lineage crown	5.36	1.72–10.25	5.10	1.71–10.01	–	–	–	–
j1: IV-a lineage crown	–	–	–	–	6.08	2.55–11.40	–	–
j2: IV-b lineage crown	–	–	–	–	5.51	2.08–10.29	–	–
k: Split between I and IV	–	–	15.34	9.19–22.53	–	–	–	–
l: Split between I and IV-a	–	–	–	–	14.54	7.84–22.69	–	–
m: Split between II–III and IV-b	–	–	–	–	15.75	10.14–23.62	–	–
n: III-2 lineage crown	–	–	–	–	6.31	3.80–9.70	–	–

HPD, highest posterior density; "–" indicates no data available, all estimated time with a unit of Ma.

*rpl*32–*trn*L data set were largely consistent with earlier findings (Drew and Sytsma, 2012) and were summarized in Table 1.

The results of the *rpl*32–*trn*L BEAST analysis suggested that *Isodon* originated ca. 27.32 million years ago (Ma) in the late Oligocene (95% HPD = 39.84–17.03 Ma; node c in Table 1 and Supplementary Fig. S2). This was highly congruent with the ages estimated by Drew and Sytsma (2012). Results obtained from the combined cpDNA BEAST analysis indicated that *Isodon* began to

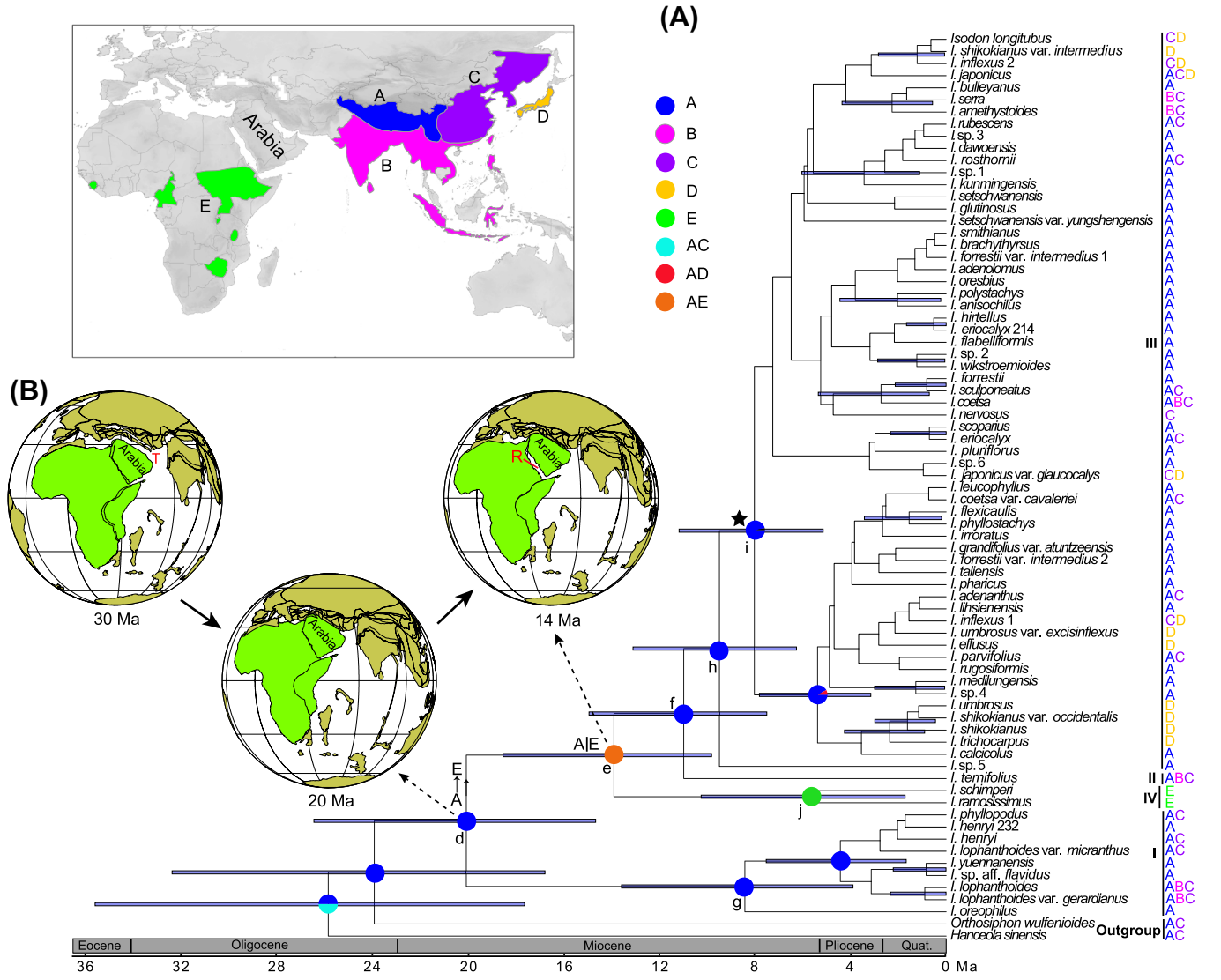

Fig. 3. Chronogram of *Isodon* derived from BEAST analysis of combined cpDNA data set (A), with the optimizations of ancestral distributions represented by the pie charts (relative frequencies of the areas) at each node, distribution of the extant species of *Isodon* and letters coding for areas are indicated on the map, the estimated dispersal events are shown beside the branches. Blue bars represent 95% credibility intervals for each node with posterior probability above 0.9; time scale is shown at the bottom. The hypothesized tectonic events occurring on the Arabian Plate are also shown (B). The star at node i indicates the shift of diversification rate based on the Relative Cladogenesis (RC) test.

diversify ca. 19.61 Ma in the early Miocene (95% HPD = 26.44–14.66 Ma; node d in Fig. 3A and Supplementary Fig. S2). The split between the African lineage and the Asian clade might occur ca. 13.61 Ma (95% HPD = 18.53–9.81 Ma; node e in Fig. 3A). The diversification of most species of *Isodon* probably occurred ca. 9.28 Ma (95% HPD = 13.09–6.26 Ma; node h in Table 1 and Fig. 3A) in the late Miocene. Results of the nrITS and *LEAFY* intron II BEAST analyses were highly consistent with that of cpDNA (Table 1), with only minor discrepancies between some nodes. Moreover, the split between clade IV and I inferred from the nrITS BEAST analysis was ca. 15.34 Ma (95% HPD = 22.53–9.19 Ma; node k in Table 1). It agreed with our cpDNA BEAST analysis. Based on the *LEAFY* intron II BEAST analysis, the split between clades I and IV-a (node l in Table 1) and the split between clades II–III and IV-b (node m in Table 1) occurred virtually simultaneously.

3.3. Diversification rates of Isodon

The LASER results rejected the null hypothesis of temporally homogeneous diversification rates and suggested variable diversification rates within *Isodon*. All of the three data sets better fitted a rate-variable model of diversification, with the diversification rate-constancy statistic ΔAIC_{RC} being 7.74 for cpDNA, 6.78 for nrITS and 11.91 for *LEAFY* intron II data set, respectively. The yule2-rate diversification model was selected as the best fitting model for all of our three data sets. The Relative Cladogenesis (RC) analyses detected significant shifts of diversification rates within *Isodon* at ca. 7.84 Ma based on cpDNA data set (node i in Fig. 3; $p = 0.002$), at ca. 9.87 Ma based on nrITS data set (node h in Supplementary Fig. S3; $p = 0.035$), and at ca. 6.31 Ma based on *LEAFY* intron II data set (node n in Supplementary Fig. S4; $p = 0.004$). These events might have resulted in the unsolved phylogenetic relationships within clade III. Our analyses of the LTT plots also supported the findings obtained from the LASER and GERGER analyses. The semi-logarithmic LTT plots derived from our three data sets clearly indicated an increase of the diversification rate (accelerated lineage accumulation) within *Isodon* after ca. 10 Ma in the late Miocene and towards the current time (Fig. 4), in contrast, relatively stable rates of diversification were illustrated before that time.

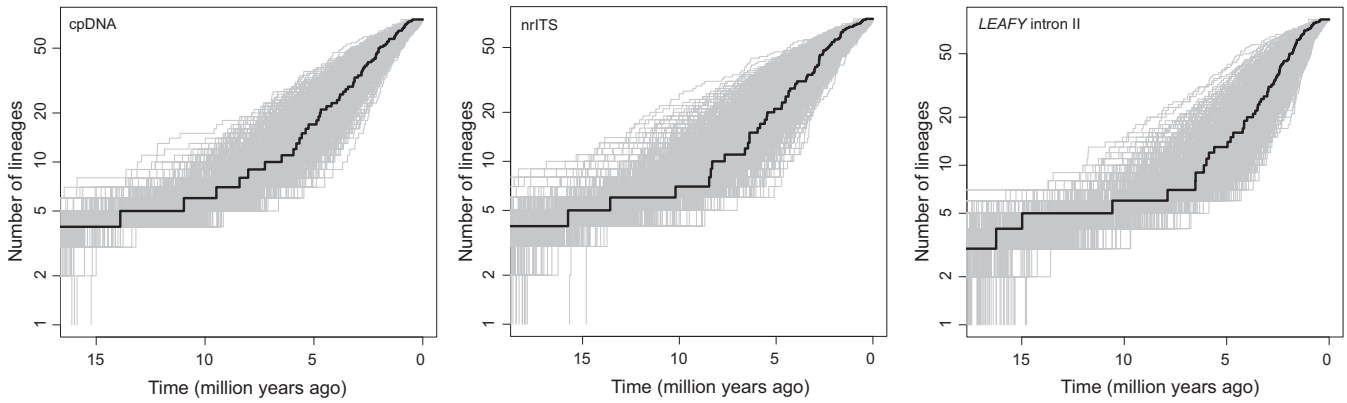

Fig. 4. Lineage through time plots (with 95% confidence intervals) of *Isodon* derived from cpDNA, nrITS and *LEAFY* intron II data sets. The bold line corresponds to the maximum credibility tree from the BEAST analyses.

3.4. Ancestral area reconstruction

The ancestral distributions and the dispersal events inferred from the S-DIVA and the Lagrange analysis produced similar results. Setting the 'Maxarea' limit to 2 and 3 provided almost identical results. In addition, the results obtained from cpDNA and nrITS data sets were highly congruent (Supplementary material, Table S5). Thus only the results with 'Maxarea' set as 2 of S-DIVA analysis based on cpDNA data set were shown in Fig. 3A.

The QTP and adjacent regions were inferred as the most likely ancestral area of *Isodon*, species occurring in Africa were estimated to have dispersed from the QTP and adjacent regions in the early to middle Miocene (ca. 20–14 Ma; nodes d and e in Table 1 and Fig. 3A). The common ancestor of clades II, III, and IV might have occurred in the QTP and adjacent regions, as well as tropical Africa. The stem lineage of clades II, III, and IV subsequently underwent a vicariant split of the combined ancestral area (node e in Fig. 3A). The ancestral area for the early-branching clade I (node g in Fig. 3A), clades II and III (node f in Fig. 3A) and clade III (node h in Fig. 3A) was also inferred as the QTP and adjacent regions.

3.5. Inference of chromosome number change

The "Const_rate_demi" model (Loglikelihood = −27.22; AIC = 60.44), with the chromosome duplication rate equal to the demi-duplication rate, was selected as the best-fitting model for the data set. The inferred change events of chromosome number within *Isodon* were shown in Fig. 5. The ancestral haploid chromosome number of *Isodon* was estimated to be $n = 12$ (probability = 0.99). Gain and demi-polyploidization (the union of a reduced and an unreduced gamete) may have been involved in the speciation of *I. lophanthoides* and the African *I. ramosissimus*.

4. Discussion

4.1. Molecular phylogeny of Isodon

Phylogenetic reconstructions using three different data sets consistently show that *Isodon* is monophyletic with four major well-supported clades (I, II, III, and IV; Figs. 1 and 2). Three of the clades (I, II, and III) recovered are consistent with previous findings (Zhong et al., 2010). In addition, the two African-endemic species, one of which has an aberrant chromosome number, formed a strongly-supported clade IV within *Isodon*. As in other putative rapid radiations (e.g. Hughes and Eastwood, 2006; Liu et al., 2006), large polytomies were found in all three gene trees in our study. A similar situation was encountered with *Solms-laubachia* Muschl. (Brassicaceae), which also has a center of diversity in the Hengduan Mountains region (Yue et al., 2009). This pattern reflects the challenge of recovering recent rapid radiations that exhibit extremely short internal branch lengths (Richardson et al., 2001; Shavit et al., 2007). Resolving the species phylogeny for a group that radiated explosively is particularly difficult, because reticulation may occur during species divergence due to incomplete reproductive isolation (see below; Rokas et al., 2003; Belfiore et al., 2008). For example, it has been suggested that ⩾25,000 bp are needed in Saxifragales to resolve a rapid radiation with high support values (Jian et al., 2008). Future research applying large-scale genomic sequence data (e.g. Fior et al., 2013) therefore may be needed to resolve species-level relationships within *Isodon*.

Rapid diversification may trigger extensive hybridization and introgression because intrinsic barriers to reproductive isolation among recently evolved lineages that prevent gene flow between many species may have had too little time to develop fully (Mallet, 2007). Indeed, within *Isodon*, seven natural interspecific hybrids have been recorded in Japan alone (Murata and Yamazaki, 1993). Our comparison between the cpDNA and nuclear DNA trees indicated that either *I. eriocalyx* or *I. phyllostachys* was the possible paternal donor of *I. eriocalyx* 214, with *I. hirtellus* as the possible maternal donor. This hybrid was more similar morphologically to *I. eriocalyx*, based on our field observations. In addition, *I. henryi* 232 was also inferred to be a hybrid between *I.* sp. aff. *flavidus* (paternal) and *I. henryi* (maternal). Our phylogenetic network analysis shows evidence for additional hybridization events (Supplementary Fig. S1B), which indicates that ongoing hybridization may play an important role in the evolutionary history of *Isodon*, and thus greatly inhibit proper classification.

In this study, the cpDNA and nrITS gene trees showed incongruent phylogenetic positions for the African clade (Fig. 1A and B). The phylogenetic network between cpDNA and nrITS also indicated that the African clade was of hybrid origin between distantly-related parent species, one from clade I and the other from clade II–III (Supplementary Fig. S1A). This result was further corroborated by the gene tree of the low-copy nuclear gene *LEAFY* intron II, which showed that two divergent homoeologous copies of the African clade grouped with putative maternal (clade II–III) and paternal (clade I) parents, respectively (clades IV-a and IV-b in Fig. 2). Based on current data, there are different plausible scenarios for the paternal (maternal) parent of the African clade. It could be the common ancestor of clade I (clade II–III), or alternatively the progenitor could have been an extinct lineage that was closely related to clade I (clade II–III). The fact that only one copy of nrITS was found indicates that homogenization to the paternal type

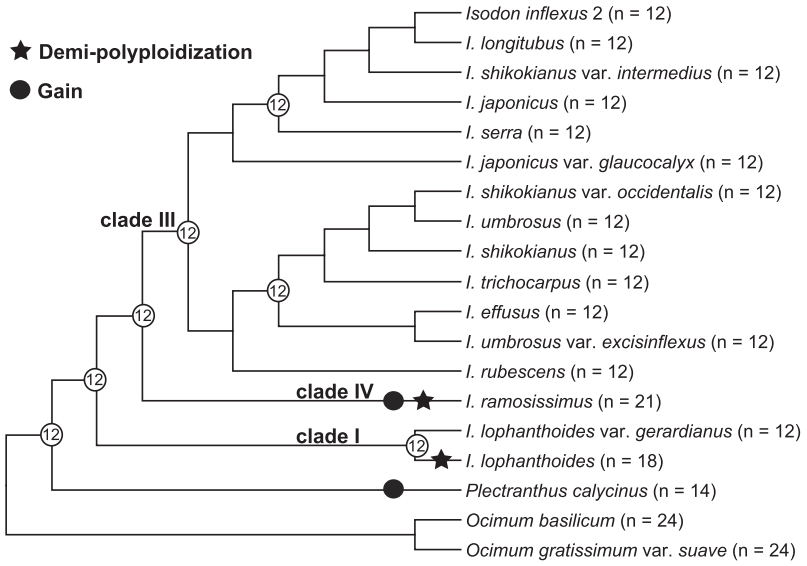

Fig. 5. Chromosome number evolution of *Isodon* inferred using chromEvol over a pruned Bayesian tree from the combined cpDNA data set. Only taxa with reported chromosome numbers are included. Inferred ancestral haploid numbers (n) are displayed in a pie chart at each node, a whole pie chart represents the haploid number with probability $\geqslant 0.99$. Different portions of the pie charts represent two or more haploid numbers with different probability values.

might have occurred in the two African species, as reported by other hybridization studies (e.g. Kovarik et al., 2005).

Analysis of changes in chromosome number based on our limited species sampling (Fig. 5) suggested that the ancestral haploid chromosome number of *Isodon* was $n = 12$ with high probability (0.99). A demi-polyploidization event was inferred to be involved in the origin of the African species *I. ramosissimus*, although the origin of the other African species can not be inferred because of missing chromosome number data. A likely formation of the inferred demi-polyploidization of *I. ramosissimus* is the fusion of two unreduced gametes to yield $n = 24$, followed by chromosome fusions (Lysak et al., 2006) or loss to yield $n = 21$. Taken together, our results from DNA sequence data and chromosome number evolution suggest that the African clade was likely formed through allopolyploidy. However, it must be noted that owing to limited information on chromosome numbers in *Isodon*, further comprehensive cytological studies are needed to confirm our findings.

4.2. Rapid radiation of Isodon in the Hengduan Mountains region

Polytomies found in all three gene trees, together with extremely short internal branch lengths, indicate that a rapid radiation might have occurred in the evolutionary history of *Isodon* (Richardson et al., 2001; Shavit et al., 2007). Additionally, our biogeographic reconstruction inferred that the QTP and adjacent regions are the most likely ancestral area (Fig. 3A), with the HMR (southeastern edge of the QTP) currently containing the greatest diversity of *Isodon* species (ca. 70%). The question raised here is this: what triggered the extensive diversification of this species-rich genus in this region?

Several studies have attempted to illustrate the relationship between geological events and the origin and diversification of organisms within the QTP area (e.g. Liu et al., 2006). However, due to the lack of convincing fossil records, divergence time estimates of previous studies have been hampered because of using only one calibration point (Liu et al., 2006), or secondary calibrations based on results obtained from previous studies (Zhang and Fritsch, 2010). Some studies have even attributed the low resolution of species-level phylogenies solely to a rapid radiation, without divergence time estimation (e.g. Lu et al., 2010). Others only use the DNA sequence substitution rate instead of fossil calibrations in their estimates of divergence times (e.g. Liu et al., 2002).

In this study, the divergence time estimation relies on two fossils of Lamiaceae that are well accepted (Harley et al., 2004; Martínez-Millán, 2010) and have been used in recent studies within the Nepetoideae (Drew and Sytsma, 2012, 2013). The results of our molecular dating suggest that the diversification of the most diverse lineage III within *Isodon* may begin ca. 9.28 Ma (95% HPD = 13.09–6.26 Ma) in the late Miocene (node h in Table 1 and Fig. 3A) based on cpDNA data set, ca. 9.87 Ma (95% HPD = 14.91–5.91 Ma) on nrITS data set, and ca. 7.62 Ma (95% HPD = 11.63–4.70 Ma) on *LEAFY* intron II data set. To confirm if the diversification rate in *Isodon* has changed over time, we conducted several analyses as implemented in packages based on R application. Rate-constancy statistic ΔAIC_{RC} calculated in LASER based on cpDNA, nrITS and *LEAFY* intron II data sets all suggested variable diversification rates within *Isodon*. The Relative Cladogenesis (RC) analyses revealed shifts of diversification rates within *Isodon* at ca. 7.84 Ma (cpDNA, Fig. 3), ca. 9.87 Ma (nrITS, Supplementary Fig. S3), and ca. 6.31 Ma (*LEAFY* intron II, Supplementary Fig. S4). The semi-logarithmic LTT plots derived from our three data sets also indicated an accelerated lineage accumulation after ca. 10 Ma (Fig. 4). These estimates correspond well with the third of four major QTP uplifts believed to have occurred ca. 22–20, 15–13, 10–8, and 3.6–0 Ma (Harrison et al., 1992; Coleman and Hodges, 1995; Shi et al., 1999; Spicer et al., 2003), although the exact timings of these uplifts are still being debated. The extensive uplift of the QTP and the aridification in this region due to the onset of the East Asian and Indian monsoon climate occurring around 8 Ma was also corroborated by researchers applying paleobotanical data (e.g. Molnar et al., 1993; An et al., 2001). The large number of arid-adapted *Isodon* species (Li, 1988) appearing at that time may also be a reflection of aridification in this region.

Combining previous findings with our results, we propose that bursts of speciation triggered by geological and/or climatic changes on the QTP may be a common phenomenon and of significant importance in generating the current biodiversity within this region. Similar scenarios were reported within Andean regions (Hughes and Eastwood, 2006; Drew and Sytsma, 2013; Hughes et al., 2013).

4.3. Mechanisms of the Asia–Africa disjunction

Our data provide evidence that the two *Isodon* species (*I. ramosissimus* and *I. schimperi*) endemic to tropical Africa (Li, 1988; Ryding, 1993; Morton, 1998) belong in *Isodon* (Figs. 1 and 2). This grouping illustrates a considerable disjunction within the genus between Asia and Africa, as well as illustrating uneven species richness between the two areas. As mentioned previously, there are four explanations usually espoused to explain Asia–Africa disjunctions: the Indian rafting hypothesis (Gondwana vicariance) (Conti et al., 2002), dispersal of a high-latitude boreotropical paleoflora (Davis et al., 2002), transoceanic long-distance dispersal (Yuan et al., 2005), and Miocene overland migration via the Arabian Peninsula (Kosuch et al., 2001; Zhou et al., 2011).

The former two hypotheses are rejected, as our divergence time estimates suggested that *Isodon* possibly originated in the late Oligocene ca. 27 Ma (95% HPD = 39.84–17.03 Ma; node c in Table 1). This time is far too young to be associated with the breakup of Gondwana, rafting of India, or the once extensive boreotropical paleoflora. Besides direct trans-oceanic dispersal between Asia and Africa (Nie et al., 2013), dispersal via Eocene–Oligocene "Lemurian stepping-stones" (Schatz, 1996) which connected India, Sri Lanka, the Seychelles, and Madagascar, has also been suggested to be a possible route for organisms with Asia–Africa disjunctions (Yuan et al., 2005). However, the above two pathways seem less likely for *Isodon* because although little is known about fruit dispersal in *Isodon*, some species in Lamiaceae with similar fruit size and characters were reported to be dispersed primarily by passive ballistics across only limited distances, with secondary dispersion by water flow (Zhou et al., 1999). Similarly, unlike the winged diaspores of *Paederia* L. (Rubiaceae) that were suggested to be adapted to dispersal by ocean currents (Nie et al., 2013), the small, dry, inconspicuous nutlets of *Isodon* lack any apparent dispersal adaptations. The absence of any obvious epizoic adaptions also means that its seeds are not likely to be dispersed by birds or consumed by primates. Hence, long-distance transoceanic dispersal seems unlikely.

Similarly, our ancestral area optimization suggests that dispersal from Asia to Africa might have occurred ca. 20–14 Ma during the early Miocene (nodes d and e in Table 1 and Fig. 3A). By that time, the Lemurian stepping-stones may have no longer existed. Furthermore, there is no evidence that *Isodon* has ever occurred on the Seychelles or Madagascar. Moreover, very few extant species of tribe Ocimeae naturally co-occur in Africa and Asia and most of those that do are very widespread and likely to have been transported anthropogenically for medicinal and/or culinary uses (e.g. *Ocimum tenuiflorum* L.). The ability to disperse over relatively short distances favors overland migration, possibly through Arabia, as the most plausible explanation for the current distribution of *Isodon*. Some species of *Isodon* currently reach as far west as Afghanistan and Pakistan and the possibility of plant dispersal across northern Africa and Arabia is also supported by fossil evidence of Meliaceae in Europe, Africa, and Asia (Muellner et al., 2006).

The species of *Isodon* occurring in Africa were estimated to have dispersed from those in the QTP and adjacent regions ca. 20–14 Ma during the early Miocene. Subsequent vicariance between the QTP and adjacent regions and Africa possibly occurred ca. 14 Ma (nodes d and e in Table 1 and Fig. 3A). Despite the uncertainty of the timing of the allopolyploidy event, our results suggest the common ancestor of the African clade most likely formed in Asia and subsequently dispersed to Africa, where it gave rise to two daughter species, perhaps using Arabia as a stepping stone. Although the estimated age of collision between the Arabian and Eurasian plates varies widely, ranging from the Eocene to the late Miocene (reviewed in Yin, 2010), our findings suggest that floristic exchange between the Asian and African continents might have been feasible since at least 20 Ma (Fig. 3B). This is consistent with previous suggestions that the collision between the Arabian plate and Eurasia and the closure of the Tethys Sea occurred ca. 20 Ma, connecting Africa and western Asia (Rögl, 1998, 1999). By integrating molecular and fossil evidence, hominoid species were suggested to have dispersed out of Africa and into Eurasia through Arabia at this time (Stewart and Disotell, 1998). A recent study also support the view of overland dispersal from Asia to Africa via southwest Asia and the Arabian peninsula in *Macaranga* Thouars (Euphorbiaceae; van Welzen et al., 2014).

Importantly, two stages were suggested to be involved in the opening of the Red Sea. The estimated age for the first movement varied from 41–34 Ma to 20 Ma onwards and the second movement was dated to 5–4 Ma onwards (Bartov et al., 1980). Moreover, a drastic rifting in the Gulf of Suez (northwest of the Red Sea) may have occurred during the middle Miocene (Garfunkel and Bartov, 1977). This raises the possibility that the continuous opening of the Red Sea may have separated the Arabian Peninsula from continental Africa ca. 14 Ma to an extent (Fig. 3B) that disrupted floristic exchange between Asia and Africa. An alternative scenario is that this vicariance was caused by climate changes occurring on the Arabian Peninsula (e.g. Menzies et al., 1992). However, no *Isodon* species and very few other species of tribe Ocimeae are currently found in Northern Africa or Arabia. This could be possibly due to changes in climate and habitats in these regions, such as the desertification of Northern Africa in the late Miocene (Schuster et al., 2006). Other members of Ocimeae only persist in areas where there is some seasonal rainfall, such as western Yemen or Oman.

Allopolyploids are known to benefit potentially from "hybrid vigor" and "intrinsic fitness advantage" which allow them to exploit habitats previously unavailable to their diploid progenitors (Stebbins, 1985). The reason why only the allopolyploid clade of *Isodon* successfully survives in Africa might be attributed to the advantages of allopolyploids. This is analogous to the diversification of allopolyploid *Nicotiana* L. section *Suaveolentes* Goodsp. (Solanaceae) species in Australia following their origin, dispersal, and then extinction in South America ca. 10 Ma, also despite the lack of any obvious long-distance diaspore characteristics (Chase et al., 2003; Ladiges et al., 2011; Marks and Ladiges, 2011). Possible causes for the uneven number of species of *Isodon* between Asia and Africa could be: i) the allopolyploid African lineage did not diversify until the beginning of the Pliocene and there might have been too little time for them to speciate further; and/or ii) there could be native African habitat competitors such as *Plectranthus* L., which occupies a similar range of habitats in Africa to *Isodon* in Asia. There are no species of *Plectranthus* in the QTP region where *Isodon* is most diverse, and only two *Isodon* species occur in tropical Africa where there are ca. 180 *Plectranthus* species.

5. Conclusions

The reconstructed biogeographic history of *Isodon* provides insights into the mechanisms triggering Asia–Africa disjunctions. *Isodon* was suggested to originate in the QTP and adjacent regions in the late Oligocene. Subsequent migration into Africa in early to middle Miocene via overland migration through Arabia was considered as the most plausible dispersal scenario. The opening of the Red Sea in the middle Miocene (ca. 14 Ma) may also have played a critical role in disrupting floristic exchange between Asia and Africa. Furthermore, allopolyploidy of the African species may have facilitated the dispersal of *Isodon* from Asia to Africa, but other organisms showing similar distribution pattern should be studied to find further evidence for the overland migration

hypothesis. In addition, a rapid radiation of *Isodon* was dated to the late Miocene, which corresponds well with one of the major uplifts of the QTP and subsequent aridification events. Combined with previous studies, our results indicate that bursts of speciation triggered by geological/climatic changes on the QTP may be a common phenomenon and of significant importance in generating the current high levels of biodiversity seen within this region.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2014.04.017.

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